

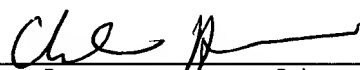
- melanocytes;
- cells of the vascular walls;
 - endothelial;
 - smooth muscle;
- epithelial cells of the respiratory tract;
- cells of the central nervous system;
- cancerous cells; and
- cells of the immune system.

REMARKS

Reconsideration of this application is requested in view of the amendments to the claims which are believed to conform to the suggestions made by the Examiner in the advisory action for which Applicants are grateful. It was indicated that the claims once corrected, would overcome the outstanding rejections. Therefore, it is believed that the application is now in condition for acceptance and favorable reconsideration of the application is requested.

Respectfully submitted,
Bierman, Muserlian and Lucas

By:


Charles A. Muserlian #19,683
Attorney for Applicants
Tel.# (212) 661-8000

CAM:ds
Enclosures

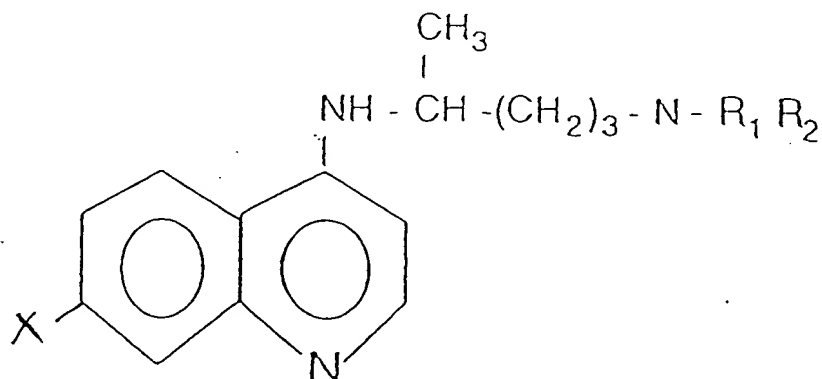
--45. A complex comprised of at least one negatively charged nucleic acid and at least one positively charged polymeric conjugate with the bond therebetween being electrostatic in nature, the polymeric conjugate containing a polylysine formed from monomers having free NH_3^+ groups,

at least 10% of free NH_3^+ groups of the said polylysine are substituted by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes,

and optionally ^{at least one} ~~some of the~~ free NH_3^+ groups of the said polylysine ^{is} ~~can be~~ substituted by a molecule with a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH_3^+ groups of the said polylysine make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residue causing destabilization of cell membrane in a weakly acid medium belong to the family of quinolines of the formula:



in which R_1 is hydrogen, R_2 is $-(\text{CH}_2)_n-\text{CO}_2-\text{H}$, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition

signal is selected from the group consisting of:

a) simple osides selected from the group consisting of α or β conformers of 2-deoxy, of 2-amino or 2-deoxy, 2-acetamido neutral monosaccharides; α or β conformers of glycuronic acid derivatives of neutral monosaccharides; α or β conformers of L-iduronic acid, of keto-deoxy-octonic acid, of N-acetyl neuraminic acid, or of N-glycoloyl-neuraminic acid; and α or β conformers of neutral 6-deoxy monosaccharides;

b) or a disaccharide selected from the group consisting of lactose and mannopyranosyl α -6-mannopyranose,

c) or complex osides selected from the group consisting of Lewis^a, Lewis^b, Lewis^x, oligomannosides and oligolactosamines ^{and d)} or peptides.

46. ~~The~~ ^{the} complex of claim 44 wherein said quinolines are selected from the group consisting of 7-chloro-4-(amino-1-methylbutylamino)-quinoline, N⁴-(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline (pyrimaquine), N⁴-(6-methoxy-8-quinolinyl)-1,4-pentanediamine, ^{and} ~~to~~ the family of pterines and ~~to the family of~~ pyridine.

47. The complex of claim 45 wherein the free NH₃⁺ groups of the polylysine are substituted with a non-charged gluconyl residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex.

48. The complex of claim 45 wherein said recognition signal

is a peptide chosen from the group consisting of

(a) anti-inflammatory peptides recognized by receptors of the vascular wall,

(b) ligand peptides of integrins,

(c) chemiotactic factors and

(d) peptide hormones.

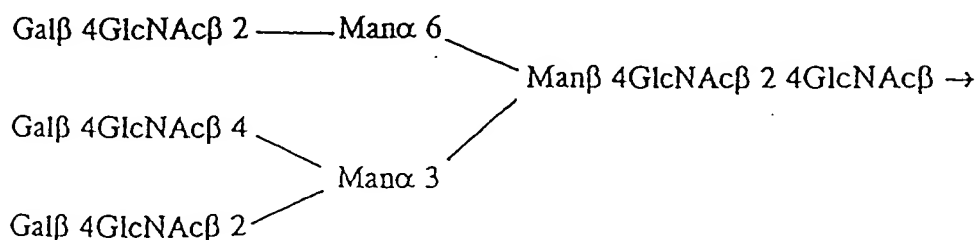
49. The complex of claim 45 wherein:

- the monosaccharide is selected from the group consisting of galactose, mannose, fucose, glucose, ribose, xylose and rhamnose and

- the oligosaccharide is selected from the group consisting of

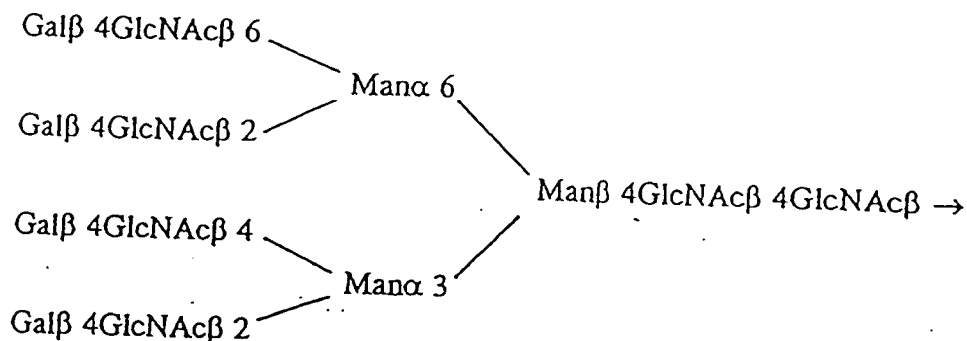
(a) Asialo-oligoside of the type of triantennar lactosamine:

the asialoglycoprotein receptor

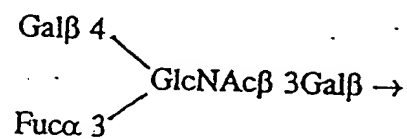


(b) Asialo-oligoside of the type of tetraantennar lactosamine ^{*the*} asialoglycoprotein

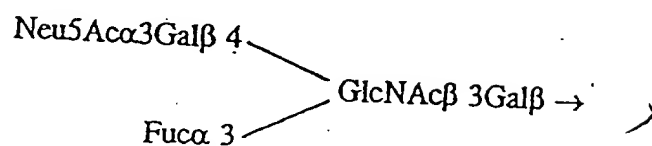
receptor



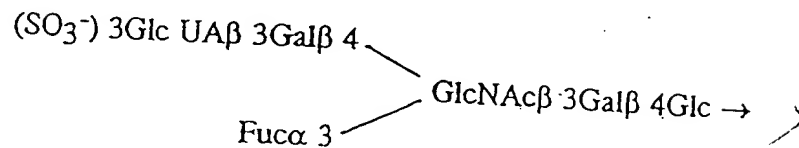
(c) Lewis x : LECAM 2/3



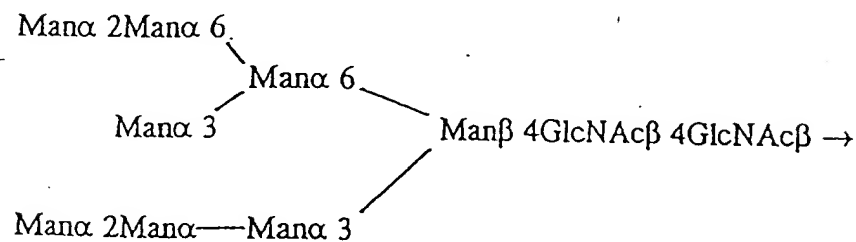
(d) Lewis x sialyl : LECAM 3/2



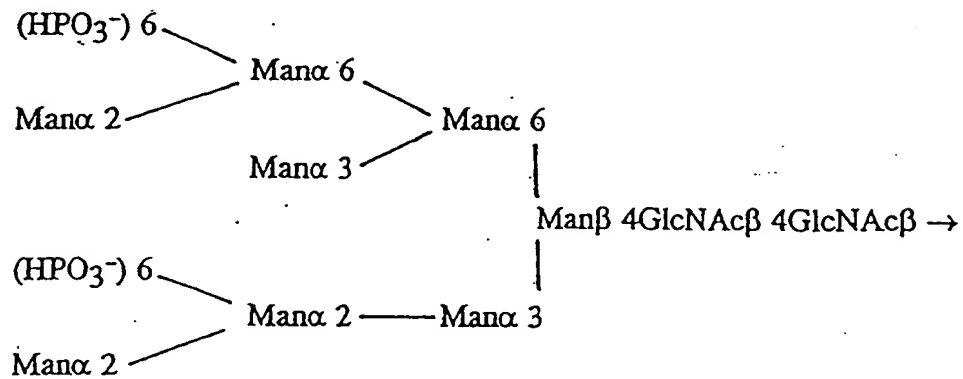
(e) Sulphated Lewis x derivative (HNK1) : LECAM 1



(f) Oligomannoside : mannose receptor

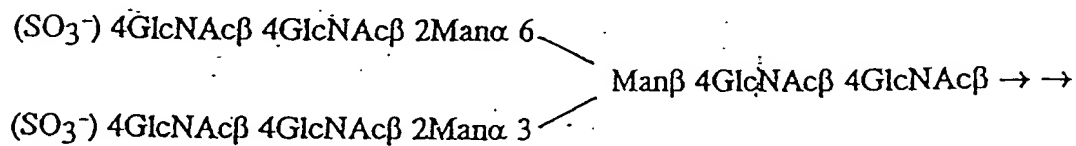


(g) Phosphorylated oligomannoside : mannose 6-phosphate receptor



(h) Oligosaccharide of the type of sulphated lactosamine:

The sulphated GalNAc 4 receptor:



- i. Lactose,
- j. $\text{Fuc}\alpha 2\text{Gac}\beta 3 (\text{fuc}\alpha 4) \text{GlcNAc}\beta 1\text{Gal}\beta 3\text{Glc}$,
- k. $\text{Fuc}\alpha 4 (\text{Ga}\beta 3) \text{GlcNAc}\beta 3\text{Gal}\beta$ and
- l. $\text{Man}\alpha 6\text{-man}$.

50. The complex of claim 49 wherein the peptides are selected from the group consisting of

- vasodilator intestinal polypeptide (VIP)
HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂
- atrial natriuretic polypeptide (ANP)
SLRRSSCFGGMRDRIGAQSGLGCNSFRY
- lipocortin

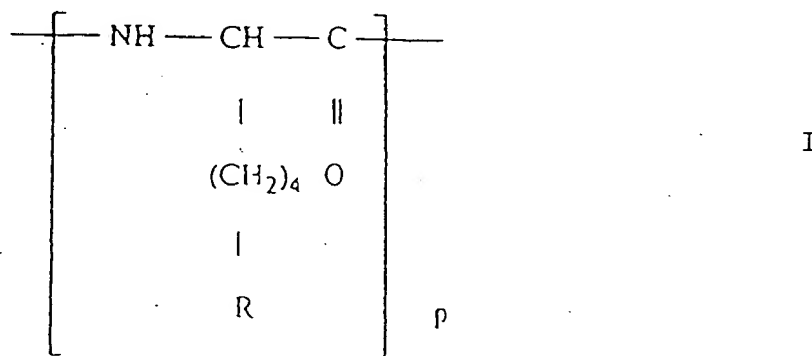
HDMNKVLDL

- bradykinin

RPPGFSPER;

of integrins, peptide hormones and chemotactic factors
peptides containing the RGC sequence, fibronectin ligand, formyl-peptides and their antagonists, α -MSH FMLP, (N-formyl-Met-Leu-Phe) and Ac-SYMEHFRWGKPV-NH₂.

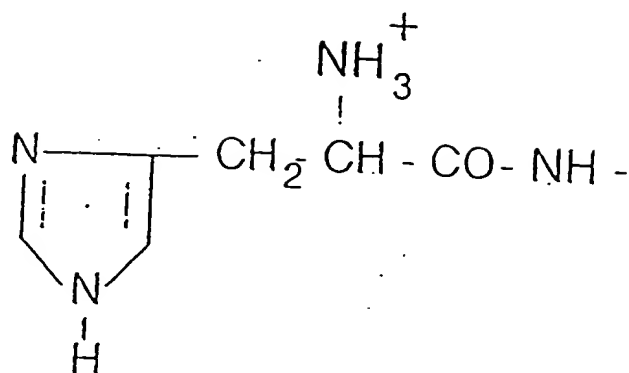
51. The complex of claim 45 wherein the polymeric conjugate has the formula:



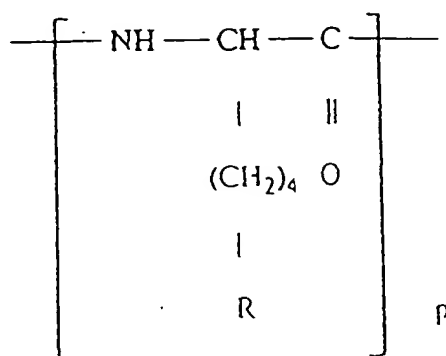
wherein:

- p is an integer from 15 to 900,
- 10 to 45% of the radical R being a residue with an imidazole nucleus,
- 10 to 90% of R being free NH₃⁺ groups,
- and optionally 0 to 45% of R being -NH-CO-(CHOH)_m-R₁, m is an integer from 2 to 15, and R₁ is hydrogen or alkyl of 1 to 15 carbon atoms.

52. The complex of claim 51 wherein R is a residue with an imidazole nucleus of the formula:

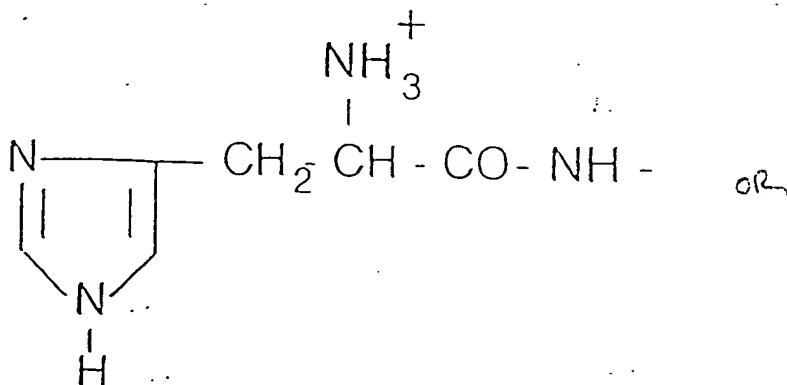


53. The complex of claim 51 wherein the polymeric conjugate has the following formula:



wherein:

- p is an integer from 15 to 900,
- 10% to 45% of R is a residue having an imidazole nucleus and optionally a free NH_3^+ , ~~it being possible for R to have~~ ^{has} the formula:



- 30% to 90% of the number of R, having free NH_3^+ , and 0 to 45% of R are substituted by a molecule which constitutes a recognition signal by a cell membrane receptor,

with the proviso that all the free NH_3^+ functions make up at least 30% of the number of monomer units of the polymeric skeleton of the above mentioned polymeric conjugate.

54. A complex according to claim 45 wherein the nucleic acid is selected from the group consisting of:

- a) marker genes and
- b) genes with a therapeutic purpose.

55. Positively charged polymeric conjugate containing a polylysine formed from monomers having free NH_3^+ groups:

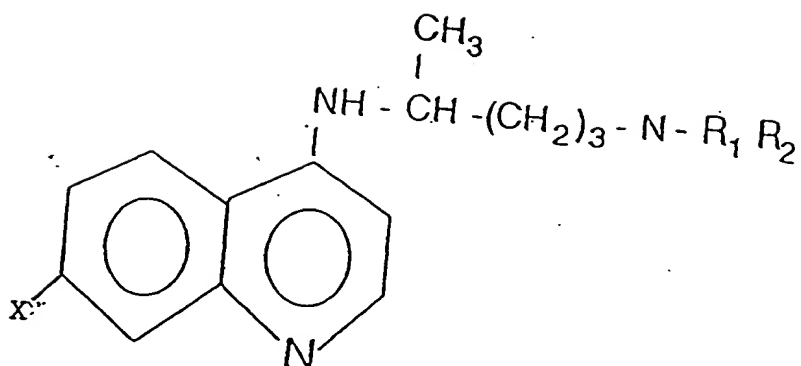
at least 10% of the free NH_3^+ groups of the said polylysine are substituted by residues which can be protonated in a weakly acid medium causing destabilization of cell membranes,

and optionally some of the free NH_3^+ groups of the said polylysine can be substituted by a molecule with a recognition signal recognized by a cell membrane receptor,

with the proviso that all the free NH_3^+ groups of the said polylysine make up at least 30% of the number of monomers of the skeleton of the polymeric conjugate,

wherein said residues causing destabilization of cell membranes in a weakly acid medium belong:

- to the family of quinolines of the formula



in which R_1 is hydrogen, R_2 is $(\text{CH}_2)_n\text{-CO}_2\text{-H}$, X is hydrogen or chlorine and n is an integer from 1 to 10, wherein said recognition signal is selected from the group consisting of:

- simple osides selected from the group consisting of α or β conformers of 2-deoxy, of 2-amino or of 2-deoxy, 2-acetamido neutral monosaccharides; α or β conformers of glycuronic acid derivatives of neutral monosaccharides; α or β conformers of L-iduronic acid, of keto-deoxy-octonic acid, of M-acetyl-neuraminic acid, or of N-glycoloyl-neuraminic acid; and α or β conformers of neutral 6-deoxy monosaccharides;

- a disaccharide selected from the group consisting of lactose and mannopyranosyl α -6-mannopyranose,

and complex osides selected from the group consisting of Lewis^a, Lewis^b, Lewis^x, oligomannosides and oligolactosamines, and peptides.

56. ^{the}Positively charged polymeric conjugate according to claim 55 wherein the free NH_3^+ groups of the polylysine are substituted with a non-charged residue causing a reduction in the positive charge of the polymeric conjugate which facilitates salting out of the nucleic acids upon dissociation of the complex, ~~the~~ said non-

charged residue being a gluconyl.

57. ~~1A~~ A composition comprising the complex of claim 45 and an inert pharmaceutical carrier.

58. A method of transfecting cultured cells comprising incubating said cells in the presence of a composition of claim 57 under conditions wherein said composition enters said cells, and the nucleic acid comprised in the complex of said composition is released *to transfect culture cells*

59. The method of claim 58 wherein the cells are selected from the group consisting of

- cells of haematopoietic strains;
- dendritic cells;
- liver cells;
- skeletal muscle cells;
- skin cells;
- fibroblasts,
- keratinocytes,
- dendritic cells,
- melanocytes;
- cells of the vascular walls;
 - endothelial;
 - smooth muscle;
- epithelial cells of the respiratory tract;
- cells of the central nervous system;
- cancerous cells; *and*
- cells of the immune system.